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Lecture 3: Genome-Wide Analysis

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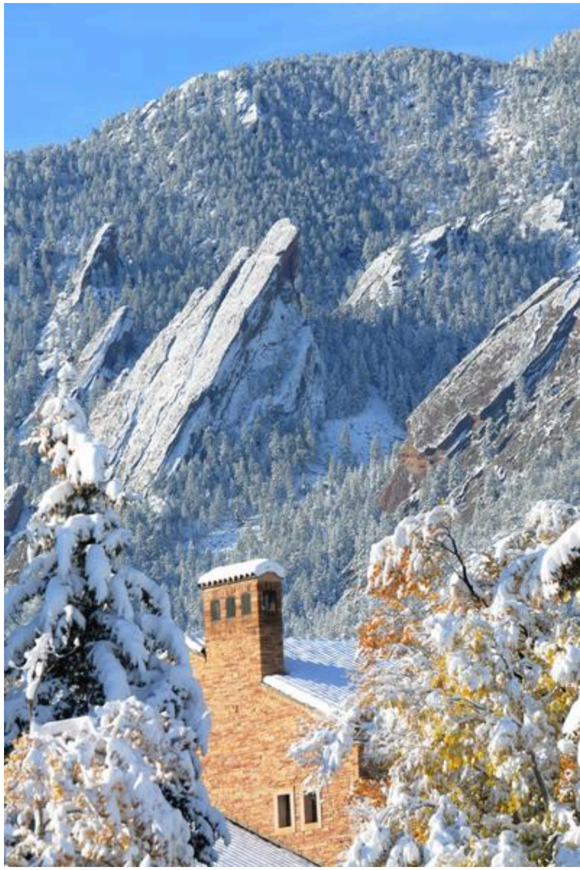

Harnessing the Information

OVERVIEW

- Study Design
- Analytic Challenges
- Analytic Considerations
- Population Stratification

Study Design

Study Design

- Often neglected in genetic research
  - See population stratification (later)
- The most popular design has been case-control studies
- However, cohort studies and family studies serve an important role

Case Control

- Dichotomous outcome
- Efficient for diseases of low prevalence
- Control selection *very* important
- Often nested within larger cohort study
- Examples
  - WTCCC
  - Psychiatric Genetics Consortium

Cohort Study

- Ideal for more common diseases/disorders
- Quantitative, discrete/binary traits
- Examples
  - Framingham Heart Study (FHS)
  - Agincourt

Family-Based

- Covered later

Analytic Challenges

Multiple Testing Multiple Testing Multiple Testing

Multiple Testing Multiple Testing Multiple Testing

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Multiple Testing Multiple Testing Multiple Testing

Multiple Testing

- GWAS
  - 1 phenotype
  - 1,000,000 markers
    - ~50,000 p-values < 0.05
- Whole Genome Sequencing
  - 1 phenotype
  - 3B base pairs
    - ?????

Addressing Multiple Testing

- Family-Wise Error Rate (FWER)
  - Bonferroni
- False Discovery Rate (FDR)
  - and variations of…
- Bayesian Approaches
  - and variations of…
- Weighted Hypothesis Testing

Dealing with Multiple Testing

- Brute Force approach comes at a cost
  - Very large samples (time/effort/resources)

- We are inherently limited in what we will be able to uncover using traditional statistical methods

GWAS to Generate Hypotheses

- No one will (or should) take a GWAS finding at face value
  - Replicate
  - Replicate
  - Replicate
- Many journals don't accept association findings without independent replication

Analytic Considerations

Coding Genotypes

- Assume a biallelic marker (SNP)
- There are three possible genotypes
  - AA
  - Aa
  - aa

Coding Genotypes

	Genotype		
	aa	*aA*	*AA*
Genotype (A)	0,0,1	0,1,0	1,0,0
Additive (A)	0	1	2
Dominant (A)	0	1	1
Recessive (A)	0	0	1

Genotype Coding

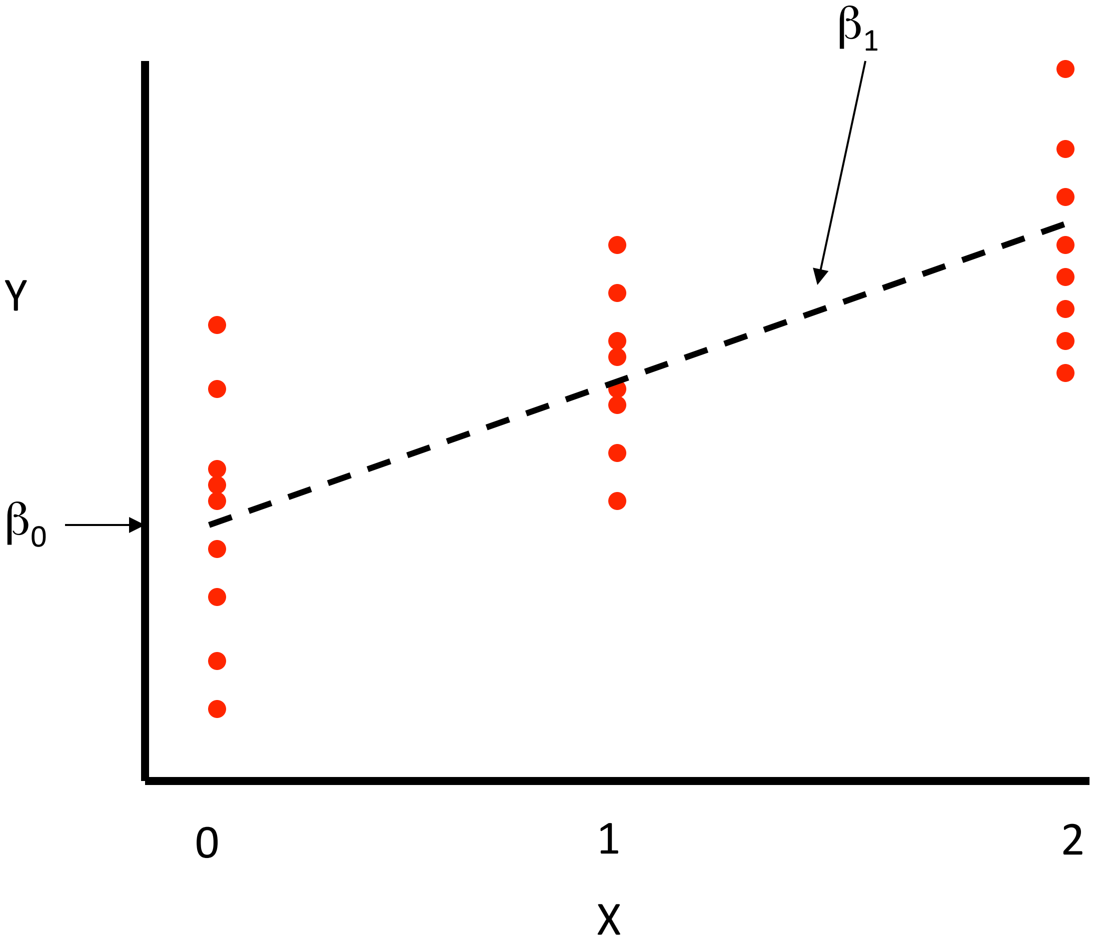
$$\text{Marker Score} = X$$

$$\text{Additive}: X = (0, 1 \text{ or } 2)$$

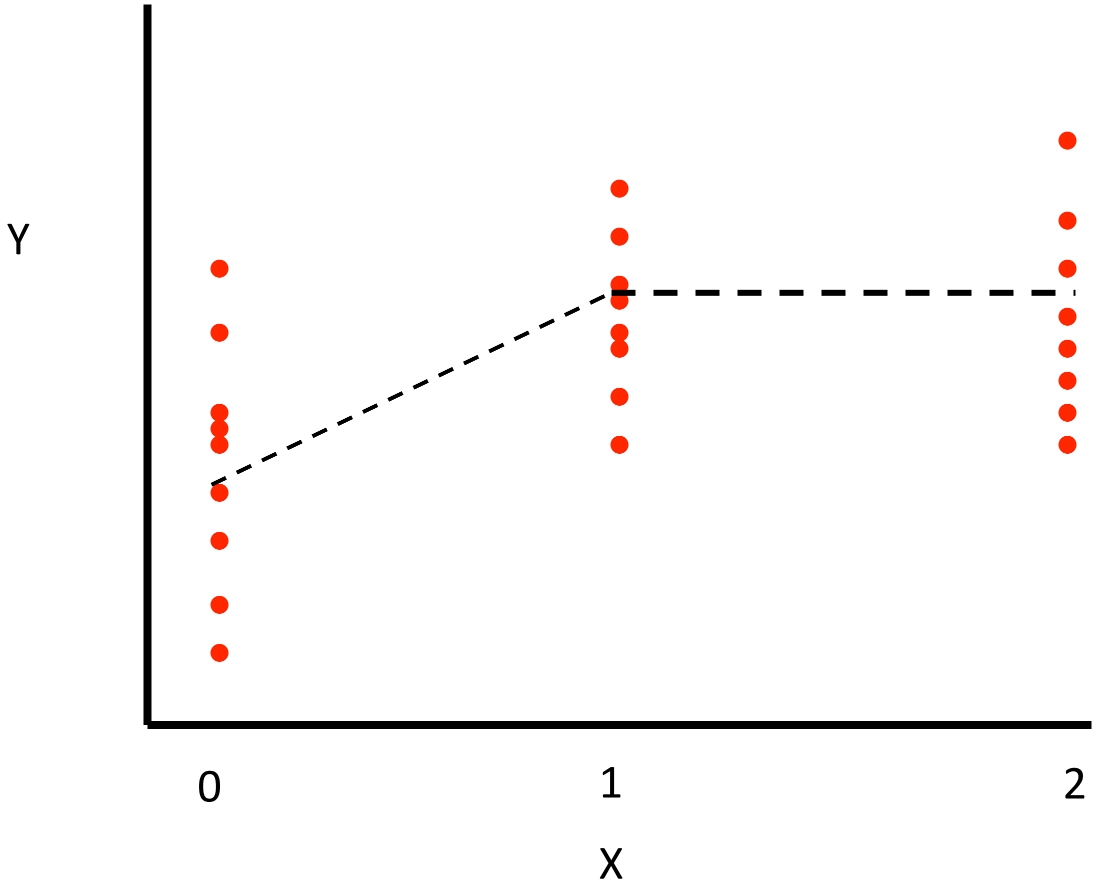
$$\text{Dominant}: X = (0 \text{ or } 1)$$

$$\text{Recessive}: X = (0 \text{ or } 1)$$

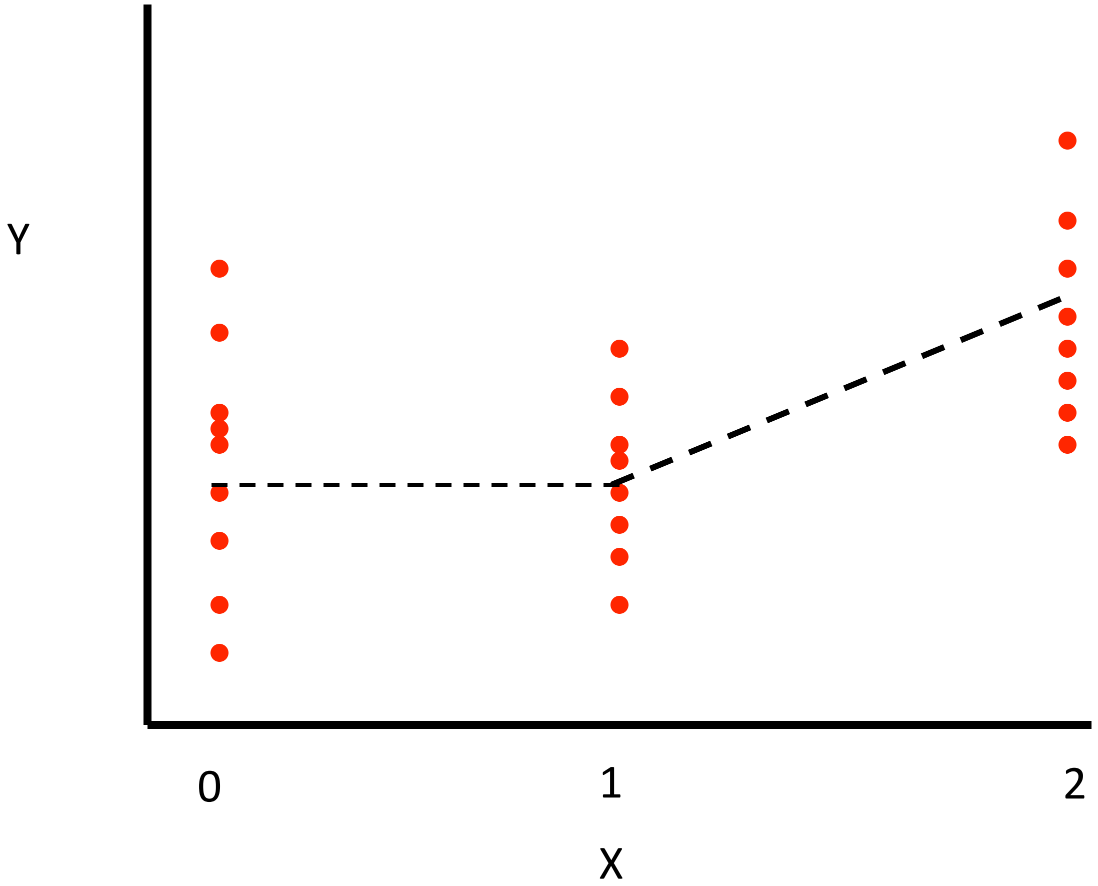
Additive Mode



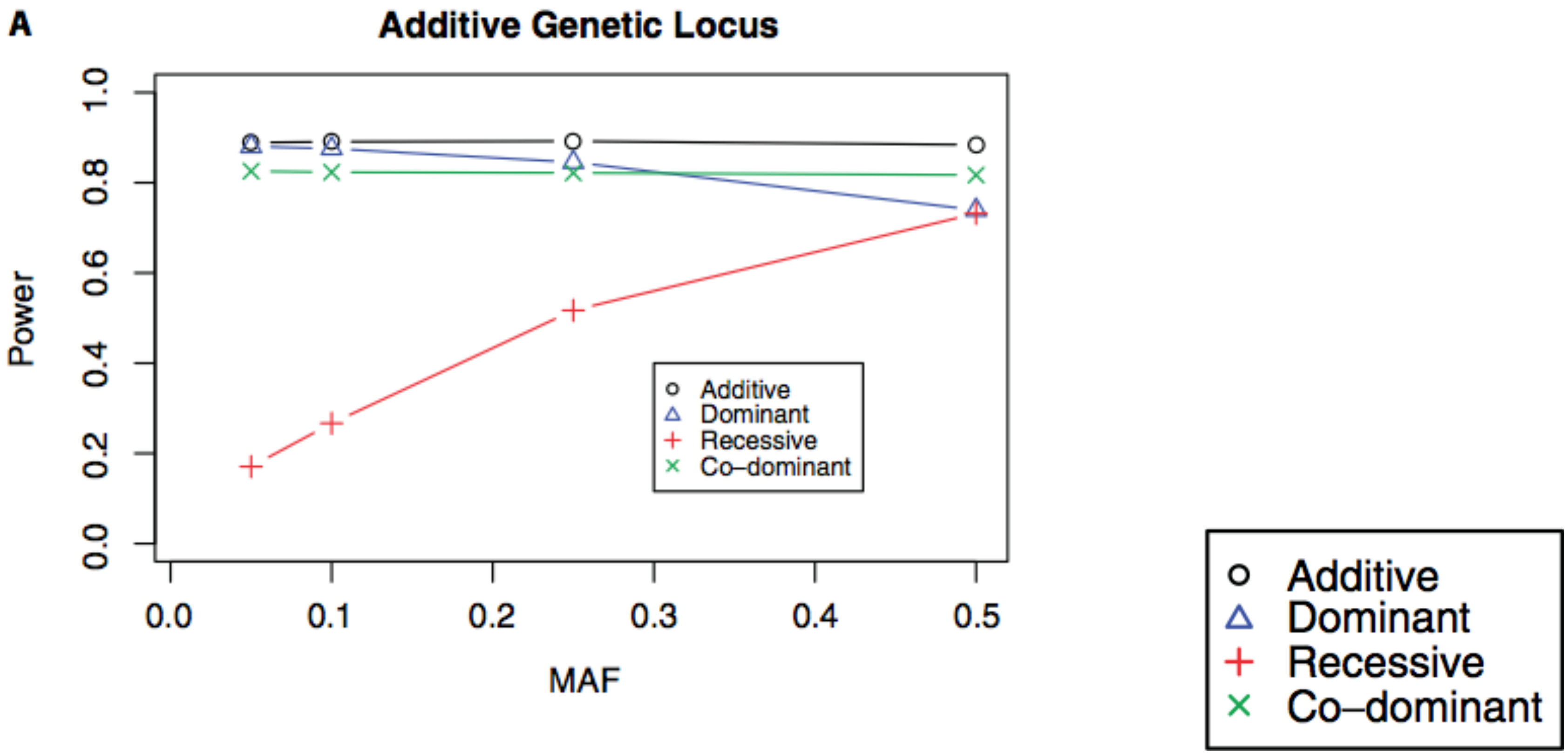
Dominant Mode



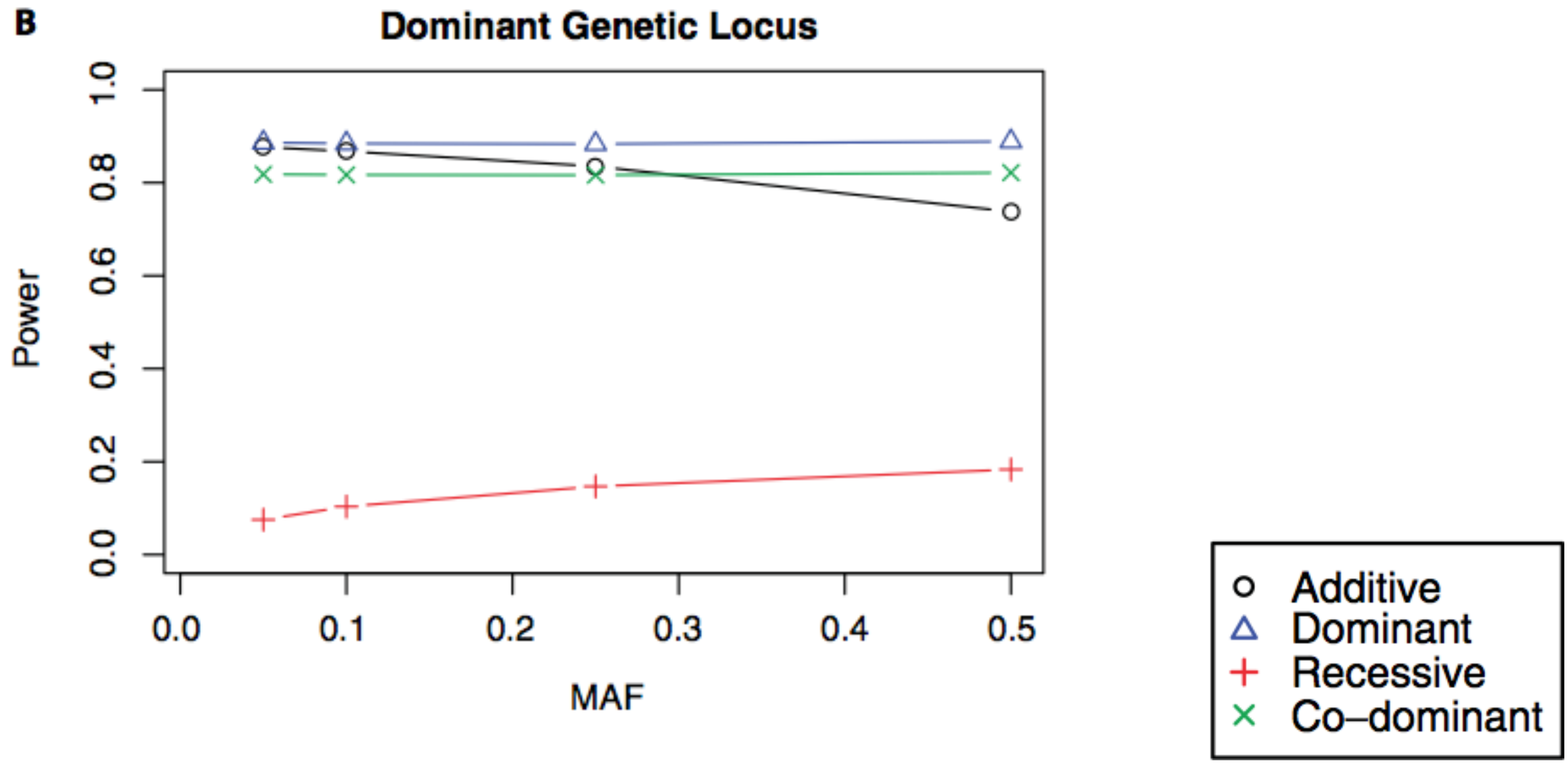
Recessive Mode



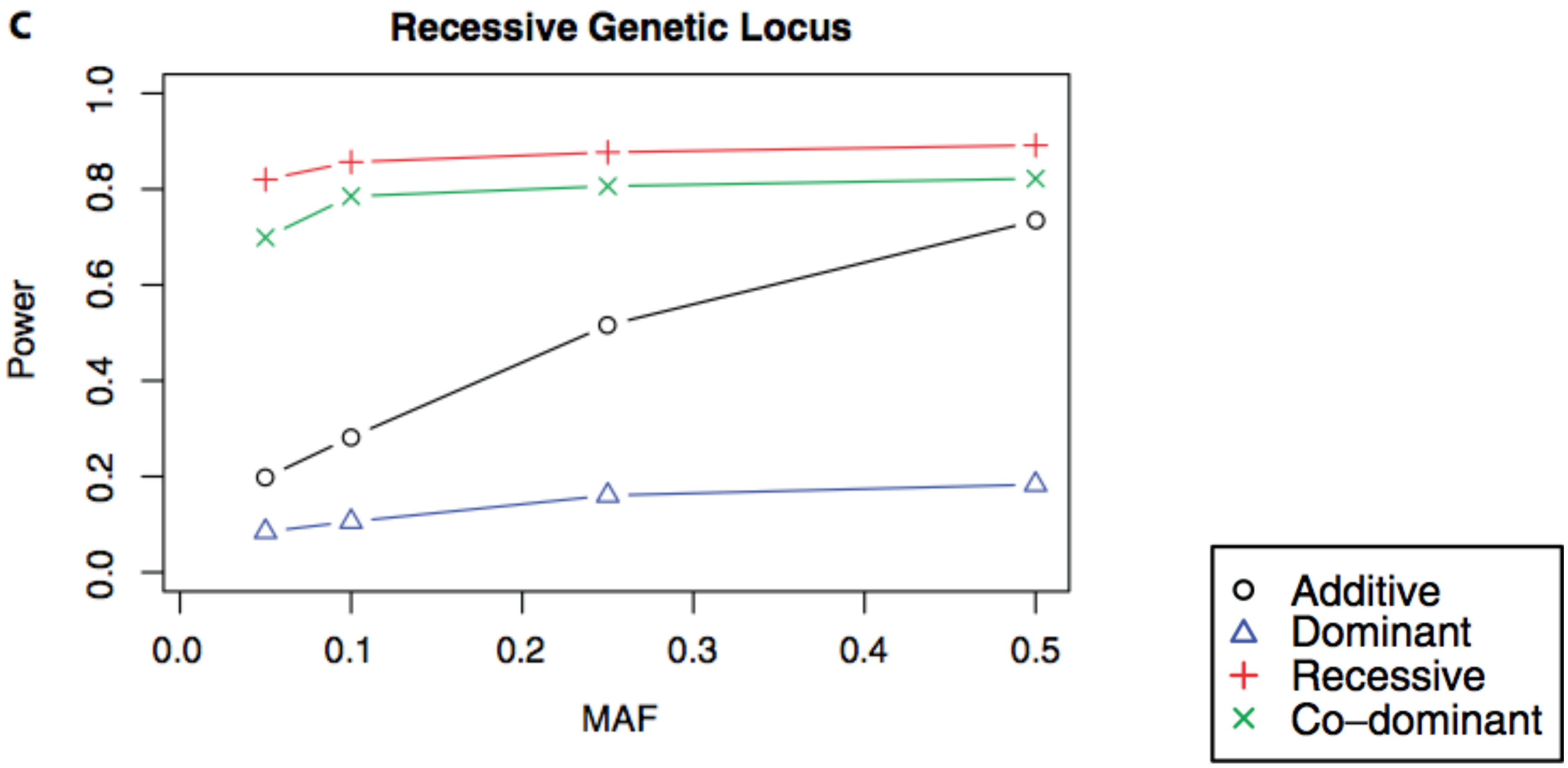
Genotype Coding…



Genotype Coding…



Genotype Coding…



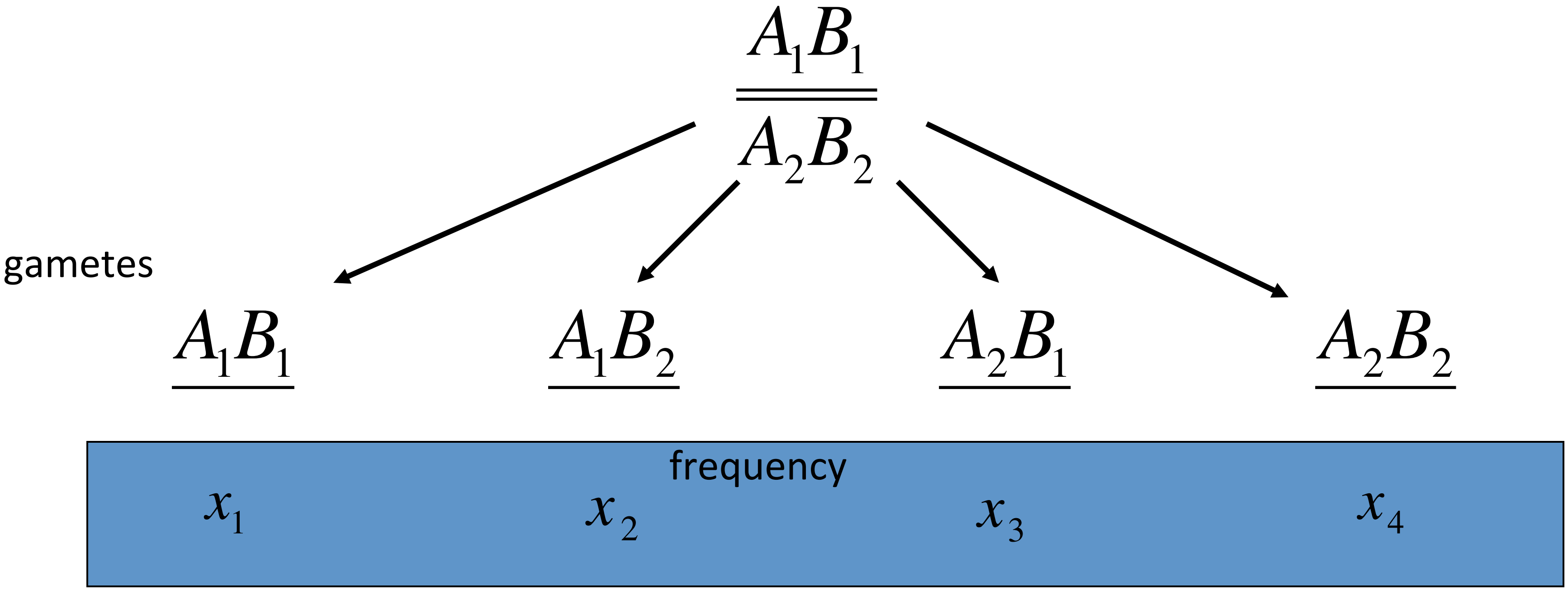
What types of analyses?

- Anything goes!
  - Typically, one large “do loop”
- Dichotomous phenotypes
- Quantitative phenotypes
- Time to onset
- Cross-sectional, longitudinal

Limitations

- Software
  - PLINK will only take you far
  - May need to write custom scripts to get what you want
    - SAS, R, SPSS, STATA, etc

Linkage Disequilibrium



$$\frac{A_1B_1}{A_2B_2}$$

gametes

$\underline{A_1B_1}$ $\underline{A_1B_2}$ $\underline{A_2B_1}$ $\underline{A_2B_2}$

frequency

x_1 x_2 x_3 x_4

Linkage Disequilibrium

Gametes	A_1B_1	A_1B_2	A_2B_1	A_2B_2
Frequency	x_1	x_2	x_3	x_4

Linkage Disequilibrium

Gametes	A_1B_1	A_1B_2	A_2B_1	A_2B_2
Frequency	x_1	x_2	x_3	x_4

Allele	A_1	A_2	B_1	B_2
Frequency	$p_{A1}=x_1+x_2$	$p_{A2}=x_3+x_4$	$p_{B1}=x_1+x_3$	$p_{B2}=x_2+x_4$

Linkage Disequilibrium

Gametes	A_1B_1	A_1B_2	A_2B_1	A_2B_2
Frequency	x_1	x_2	x_3	x_4

Allele	A_1	A_2	B_1	B_2
Frequency	$p_{A1}=x_1+x_2$	$p_{A2}=x_3+x_4$	$p_{B1}=x_1+x_3$	$p_{B2}=x_2+x_4$

D = Observed - Expected

$$D = x_1 - p_{A1}p_{B1}$$

$$D = x_1 - (x_1 + x_2)(x_1 + x_3)$$

$$\boxed{D = x_1x_4 - x_2x_3}$$

Linkage Disequilibrium

After one generation of random mating:

$$x_1' = x_1 - \theta D$$

$$x_2' = x_2 - \theta D$$

$$x_3' = x_3 - \theta D$$

$$x_4' = x_4 - \theta D$$

$$D_{t=1} = x_1'x_4' - x_2'x_3'$$

$$D_{t=1} = (1-\theta)D$$

After *t* generations:

$$D_t = (1-\theta)^t D_0$$

What does this mean?

$$D_t = (1-\theta)^t D_0$$

D_0	*theta*	*t*	*D*
1	*0.5*	*10*	*0.001*
1	*0.1*	*10*	*0.35*

Normalized LD Parameters

$$\boxed{D' = \frac{D}{D_{\max}}}$$

D_{max} $= min(p_{A1}p_{B2},p_{A2}p_{B1})$ *if D is positive*

$= min(p_{A1}p_{B1},p_{A2}p_{B2})$ *if D is negative*

Now, LD ranges from -1 to +1

r^2

Another commonly used LD measure

$$\boxed{r^2 = \frac{D^2}{p_{A1}p_{A2}p_{B1}p_{B2}}}$$

Reasons for LD

- Mutation
- Population Subdivision
- Genetic Drift
- **Lack of Recombination**
- Selection
- Non-random Mating

LD in GWAS

- SNP markers that are in close proximity may be picking up the same signal
- One typically sees a cluster of significant p-values around a signal
- Two SNPs associated

Population Stratification

Genetic Associations

- Truth
  - Causal locus (direct)
  - In LD with causal locus (indirect)
- Chance
  - If you test 100 times ~ 5 tests < 0.05
  - The association is due to chance - no causal underpinning
- Bias
  - Association is not causal
  - Population stratification

Stratification

- Essentially a confounder!

- How does it happen?

How Does it Happen?

- Two Necessary Components:
  - Subpopulation 1 has higher prevalence (mean) of disease
  - Subpopulation 1 has different allele frequency

Examine the Data

- Allele frequencies in ethnic subgroups
- Prevalence (means) in ethnic subgroups

Famous Example

Knowler et al (1988)

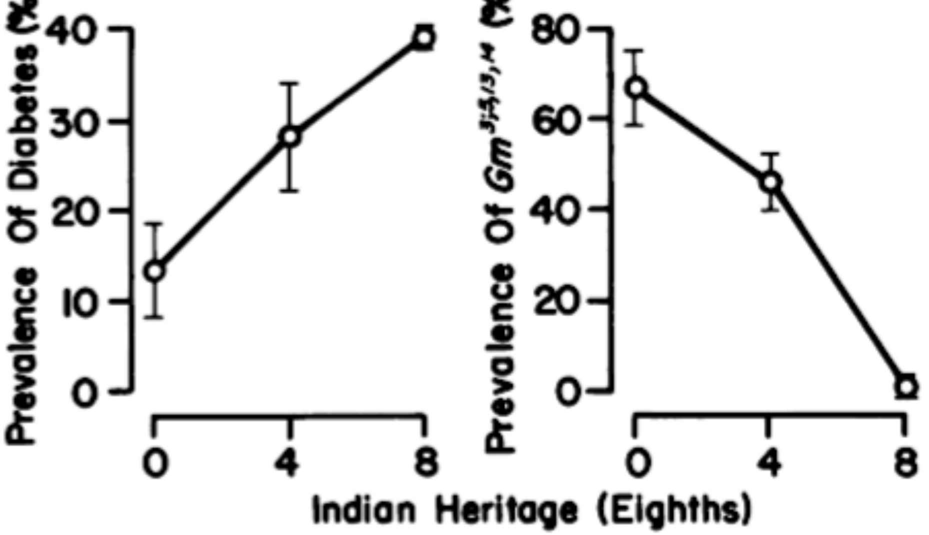
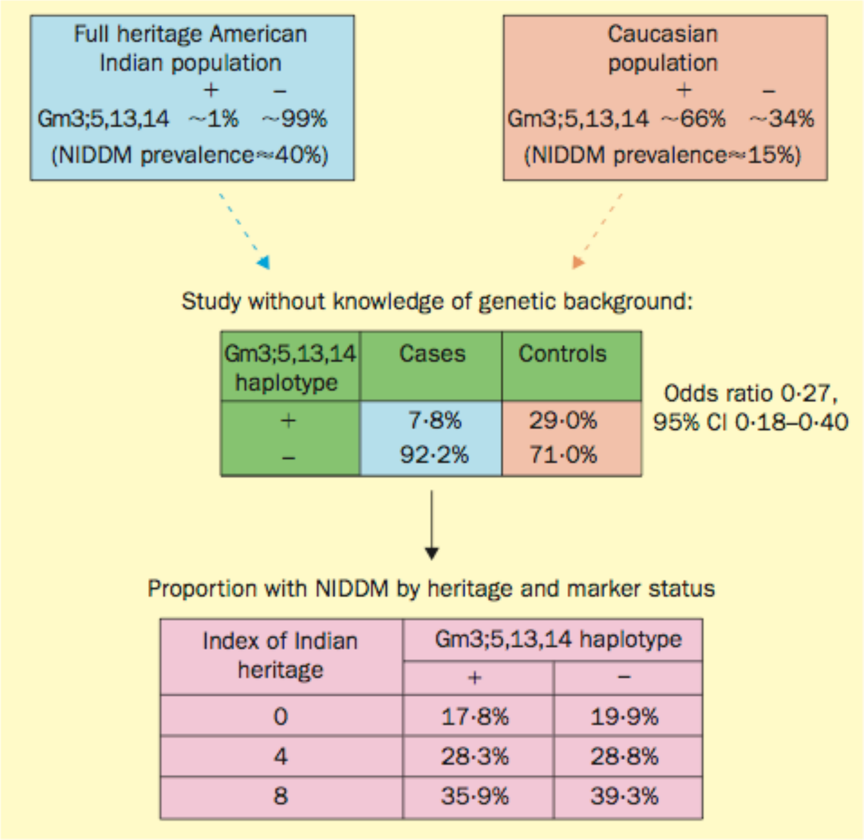


Figure 3 Age-adjusted prevalence (±1 standard error) of diabetes (left) and of $Gm^{3;5,13,14}$ (right), according to Indian heritage, among residents of the Gila River Indian Community.

Cardon et al (2003)



Full heritage American Indian population

	+	–
Gm3;5,13,14	~1%	~99%

(NIDDM prevalence≈40%)

Caucasian population

	+	–
Gm3;5,13,14	~66%	~34%

(NIDDM prevalence≈15%)

Study without knowledge of genetic background:

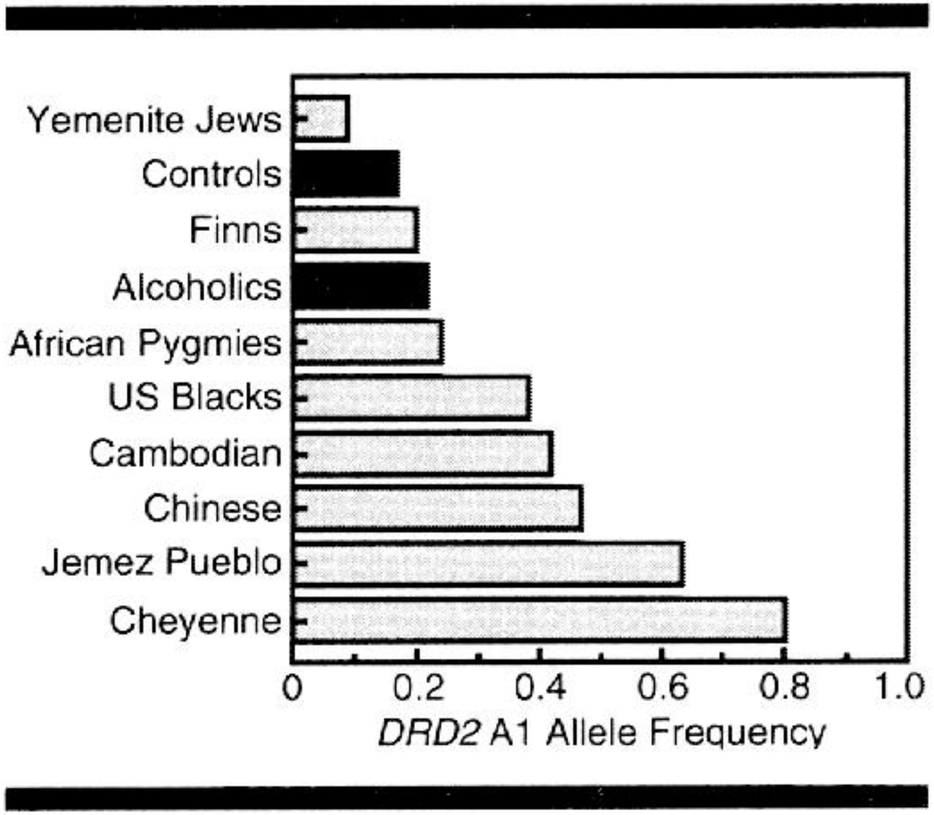
Gm3;5,13,14 haplotype	Cases	Controls
+	7·8%	29·0%
–	92·2%	71·0%

Odds ratio 0·27, 95% CI 0·18–0·40

Proportion with NIDDM by heritage and marker status

Index of Indian heritage	Gm3;5,13,14 haplotype	
	+	–
0	17·8%	19·9%
4	28·3%	28·8%
8	35·9%	39·3%

Dopamine Receptor D2



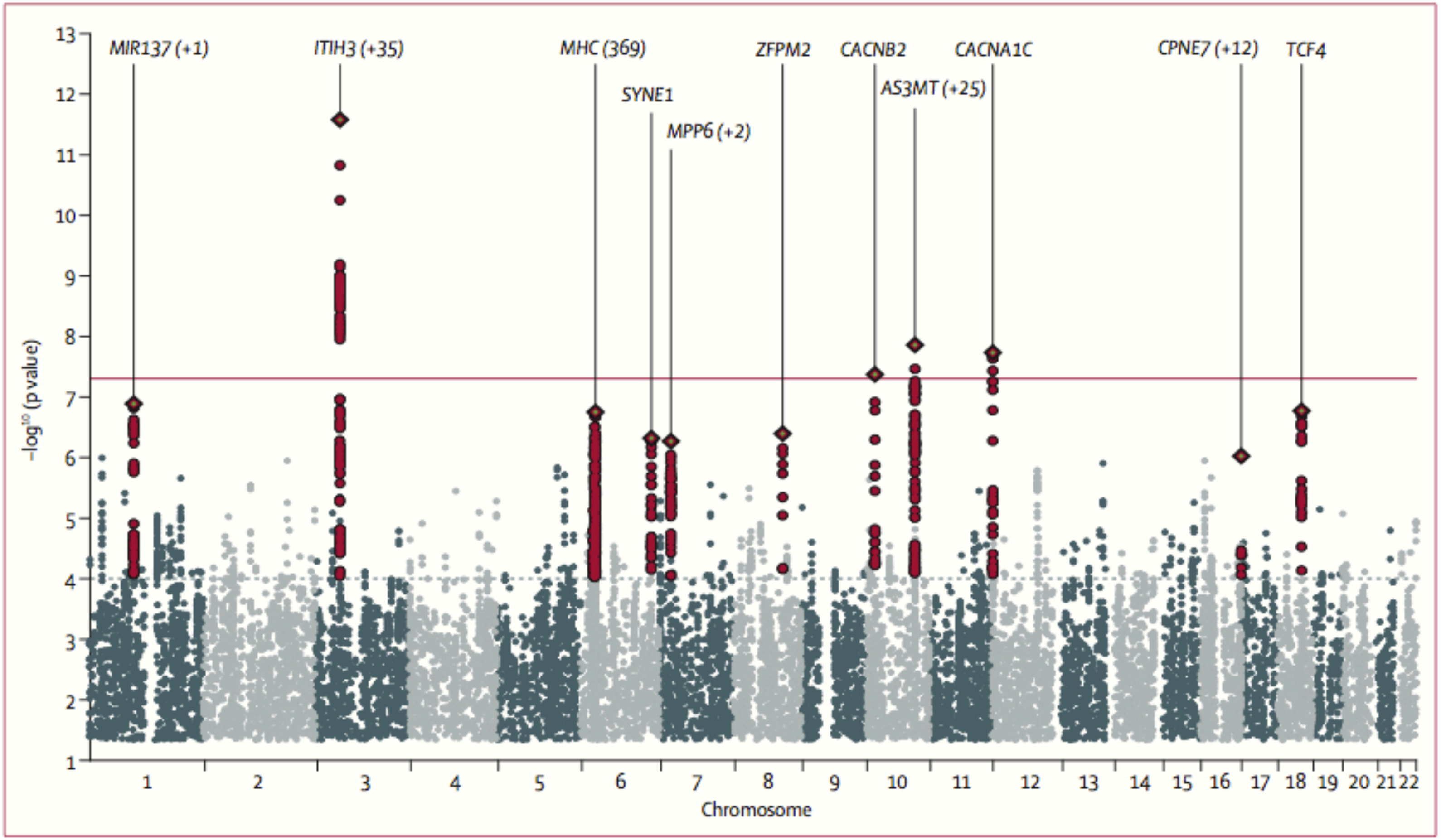
Managing Population Stratification

- Self-Reported Ancestry
  - Match (design) or Adjust (analysis)
- Use other genetic markers (ancestry informative)
  - Genomic Control (Devlin – U of Pittsburgh)
  - STRUCTURE (Pritchard – U of Chicago)
  - Eigenstrat (Reich – Broad Institute/Harvard)
  - Multi-dimensional scaling (MDS – PLINK)
- Use a family-based design

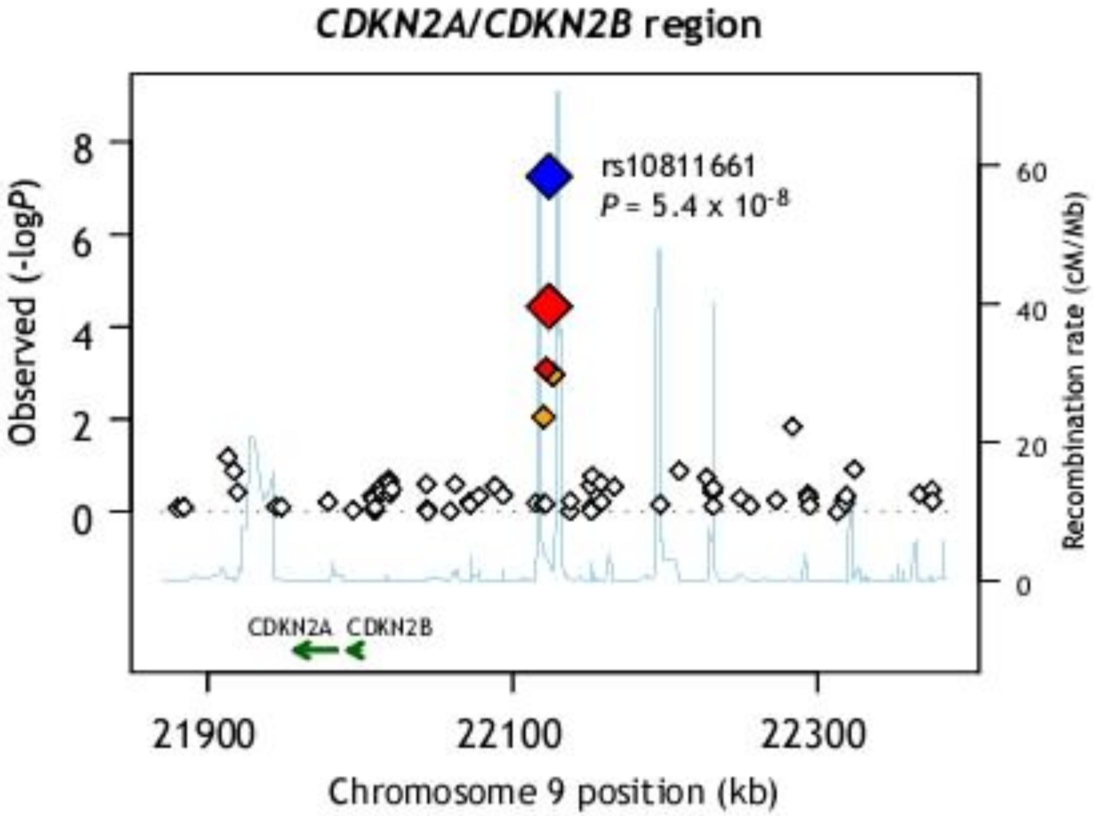
Displaying GWAS Results

- Typically, investigators will graphically display results using a Manhattan Plot
- If there is an interesting signal, investigators might also generate a regional plot
- They will also generate a quantile-quantile (QQ) plot to inspect results

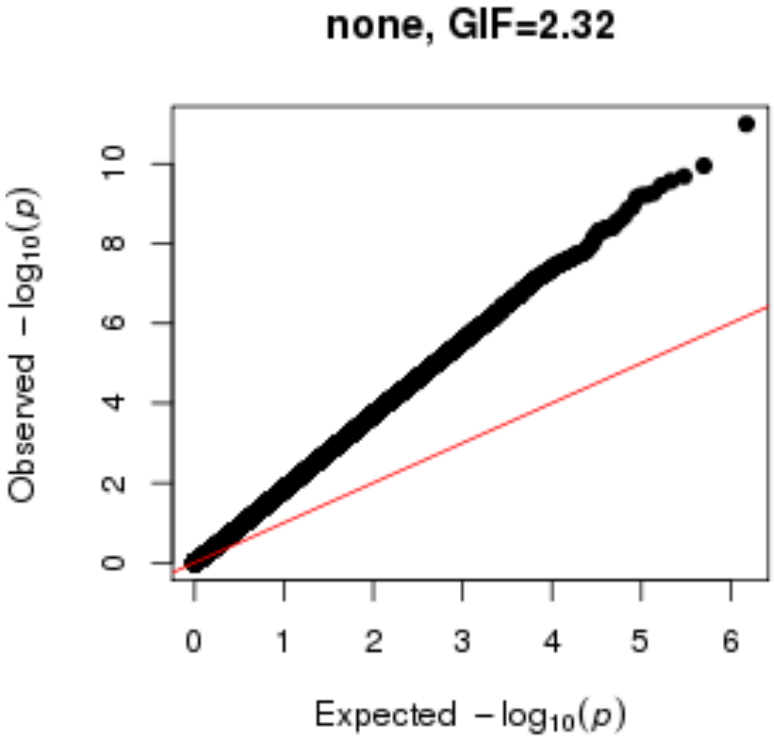
Manhattan Plot



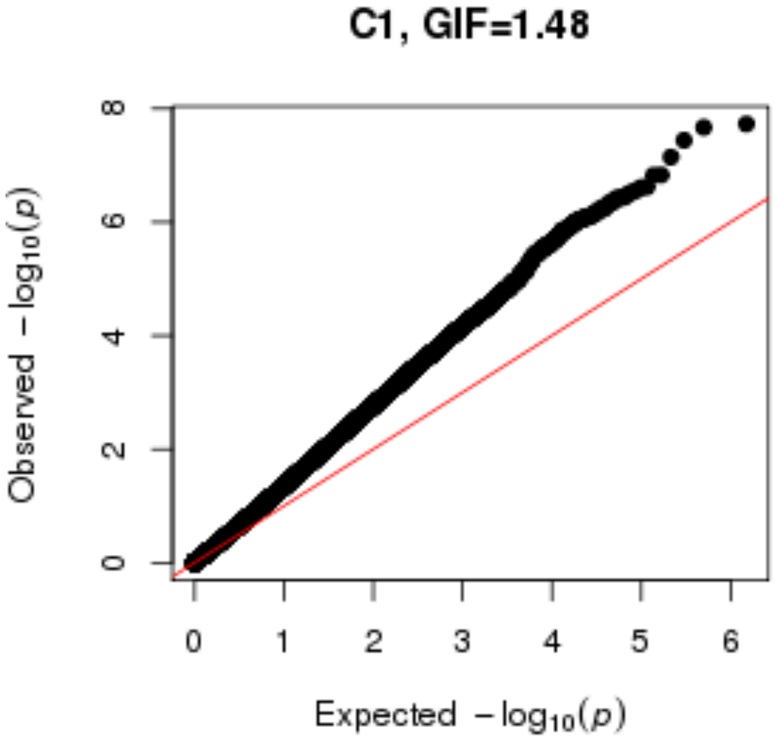
Regional Plot



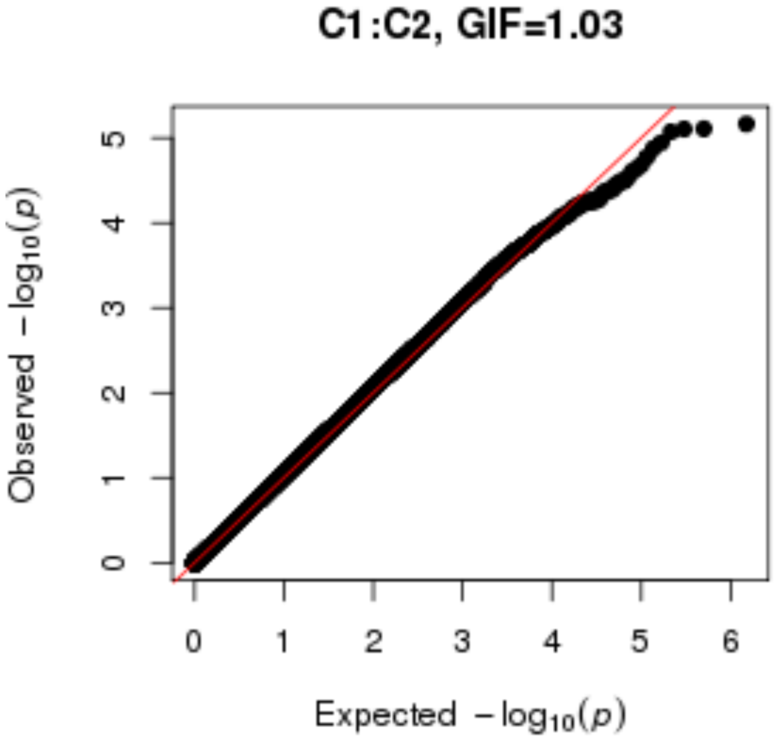
QQ Plot (unadjusted)



QQ Plot (adjusted for 1 PC)



QQ Plot (adjusted for 2 PCs)



Next up…

- Tutorial 3